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LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS RÉCEVE
A MAP OF THE ASCENDING CATECHOLAMINE SYSTEMS FOR SELF-STIMULATION IN THE RAT

Dale Corbett

A Thesis
in
The Department
of
Psychology

Presented in Partial Fulfillment of the Requirements for the degree of Doctor of Philosophy at Concordia University Montréal, Québec, Canada

July, 1978

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ABSTRACT
A MAP OF THE ASCENDING MATECHOLAMINE SYSTEMS FOR SELF-STIMULATION IN THE RAT

Dale Corbett, Ph.D.
Concordia University, 1978

The relation between sites supporting electrical self-stimulation of the brain and the location of the brainstem noradrenergic and dopaminergic systems was studied in 110 male rats of the Sprague-Dawley strain. A moveable electrode was used to test multiple sites along a single electrode penetration. Electrode placements were verified using the glyoxylic acid histofluorescence method.

Self-stimulation was not obtained from the locus coeruleus; instead, repeated stimulation at sites in or adjacent to the locus coeruleus sometimes produced a behavioral syndrome that was characterized by increased fearfulness. In other regions of the pons and midbrain, self-stimulation was not restricted to the boundaries of the noradrenergic systems. High self-stimulation current thresholds were often associated with areas rich in noradrenergic neurons while low self-stimulation thresholds were often associated with areas containing
few noradrenergic neurons. These results suggest that noradrenergic systems do not mediate brain-stimulation reward. A system of gustatory-visceral fibers arising from the solitary nucleus was suggested as a possible candidate system for the self-stimulation previously attributed to activation of noradrenergic neurons.

High rate, low threshold, self-stimulation was obtained from the A10 dopaminergic cell group and at sites along the trajectories of the midbrain dopaminergic systems. Neither the A8 nor the caudal portion of the A10 cell groups supported self-stimulation. Anterior-medial A9 electrode placements supported self-stimulation while some posterior-lateral placements did not.

In summary, the present data would appear to exclude a role for noradrenergic systems in brain-stimulation reward while they support the claim that the dopaminergic systems are involved in self-stimulation and other rewards. It was suggested that dopamine and other neurotransmitters such as serotonin may modulate goal-directed behavior by interacting with specific sensory systems such as the gustatory-visceral fiber systems.
ACKNOWLEDGMENTS

I would like to thank Dr. Roy Wise for his encouragement and support of this research and for many things too numerous to mention.

Dr. Jane Stewart has greatly enriched my student life as she has for countless other students at Concordia. I thank her for many stimulating discussions and helpful comments on this manuscript.

Dr. Peter Shizgal has encouraged this work and has always inspired me by his own example. I look forward to continued association with him.

George Fouriezos, my closest friend, deserves special thanks. He has inspired, defended, encouraged, and criticized my research at all the appropriate times, besides assisting me with any problems that hindered my progress. I am indeed fortunate to have such a friend and a colleague.

Joan Spindler has been a good friend and has taken care of many of the daily problems that face a graduate student. Without her help my task would have been much more difficult.

To my other associates, Harriet, Rudi, Gary, and others in the department, I thank you for
contributing to the pleasant and stimulating atmosphere that I have found at Concordia.

Thanks to Shelley Green for expert typing of this manuscript.

Finally, I wish to thank my wife, Penny, without whose help this work may never have been realized. It is to her that I dedicate this thesis.

This research was supported by the National Research Council of Canada, the National Institute of Drug Abuse of the United States and the Government of Québec.
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In 1954, Olds and Milner reported that electrical stimulation of certain regions of the rat brain resulted in the animals returning repeatedly to the region of the test chamber where the brain stimulation was delivered. They also trained their subjects to press a lever that delivered the stimulation to their brains via a chronically implanted electrode. These observations suggested that the brain stimulation produced a positive affective state by activating the same neural circuitry that is activated by conventional rewards such as food, water and sex. The self-delivery of brain stimulation, or intracranial self-stimulation (ICSS) as it is now called, has been demonstrated in a wide variety of species including man. While it is clear that this brain stimulation has powerful motivating effects on behavior, it is not clear what specific processes are being activated by the stimulation. It is possible that it arouses specific sensations such as tastes or odors, or instead, it may produce a general feeling or state of well being. Subjective accounts of brain stimulation in man tend to support the latter notion,
although only a few brain areas have been tested (Heath, 1964).

A principal goal of the early ICSS studies was to attempt to identify specific neural systems that supported ICSS. The initial ICSS mapping studies of Olds and his colleagues (Olds, Travis and Schwing, 1960; Olds and Olds, 1963) found ICSS to be readily obtainable throughout much of the rat brain; the limbic structures and the medial forebrain bundle being particularly effective as ICSS sites. These studies were complemented by pharmacological studies in which attempts were made to identify the neurochemistry of the ICSS substrates.

The advent of modern histochemical techniques has tended to unite the previously disparate neuroanatomical and neuropharmacological approaches to the study of the ICSS substrates. These new histochemical methods allow visualization of certain neural pathways on the basis of their neurotransmitters. Thus, a convergence of pharmacological and anatomical evidence can be directed at assessing the role of particular systems, such as the monoamine systems, in ICSS.
While general agreement does not exist about the critical neural systems underlying ICSS, the central noradrenergic (NA) and dopaminergic (DA) systems collectively known as the catecholamine systems, have received the most experimental attention (German and Bowden, 1974; Wauquier and Rolls, 1976; Hall, Bloom, and Olds, 1977). The view that ICSS results from the direct activation of one or more of the catecholamine systems will hereafter be referred to as the catecholamine hypothesis of ICSS. While this hypothesis was formally stated in its current form by German and Bowden (1974) its origin stems from the efforts of numerous investigators all of whom suggested some role for the catecholamines in ICSS (Dreese, 1966a, b; Crow, 1972, 1973, 1976; Stein, 1962, 1964; Stein and Wise, 1969, 1973; Poschel and Nintenmann, 1963). The catecholamine hypothesis of ICSS is the subject of the present investigation. The pertinent pharmacological and anatomical data will be discussed under separate headings.

Pharmacology of ICSS

Drug effects on ICSS are most commonly scaled in terms of changes in ICSS rates (e.g. lever
pressing) or ICSS current thresholds. It has been widely assumed that ICSS rates and thresholds are measures of the reward value or reward magnitude of the brain stimulation. Before proceeding to examine specific drug effects on ICSS it is first necessary to describe the assumptions behind the use of rate and current threshold measures. High rates of lever pressing have been interpreted by some as indicating that the brain stimulation has a high reward value, whereas low rates of lever pressing are interpreted as indicating a low reward value. These interpretations, though perhaps correct, are not the only possible interpretations (Valenstein, 1963). Response rates are also determined by a number of other factors such as the response topography and the general health of the animal. That is, the particular motor responses inadvertently elicited by the brain stimulation may impair the animal's ability to lever press at a high rate. Moreover, Hodos and Valenstein (1962) have shown that rats implanted with several ICSS electrodes do not necessarily choose the electrode that yields the highest rates of ICSS. Despite these considerations, response rates continue to be
widely used in ICSS studies.

The other dependent measure frequently employed in ICSS studies is an index of reward magnitude, which is the current threshold. This measure is the current at which ICSS rates fall below some predetermined level. The level chosen may be a percentage of the animal's maximum possible rate or it may be some fixed rate of responding (e.g., 20 lever presses per min.). While no single definition of current threshold is adhered to, it may be defined as the current intensity that is by some degree, less than the current intensity required to maintain responding at a particular criterion level. The current threshold seems a better measure of reward than response rates since the current threshold is less affected by response topography and other rate-limiting factors discussed above. With the rate measures and current thresholds defined, the pharmacological data bearing on ICSS can now be discussed.

The first indication that the catecholamines might be critically involved in ICSS came from the pharmacological studies of Olds and associates (Olds, Killam, and Bach Y Rita, 1956; Olds and
Travis, 1960) who showed that low doses of chlorpromazine, a catecholamine receptor blocker, reduced lateral hypothalamic ICSS rates without causing obvious sedative effects. Stein (1962) replicated the above finding and went on to demonstrate that no ICSS occurred after reserpine pretreatment, a drug that depletes norepinephrine, dopamine and serotonin stores. Furthermore, ICSS seemed to be particularly sensitive to the effects of central nervous system stimulants such as the amphetamines. The general pharmacological profile of ICSS that emerged from these early studies and numerous others which followed (see German and Bowden, 1974; Olds, 1976) was that drugs that prolonged or increased the synaptic action of the catecholamines both increased ICSS rates and decreased ICSS current thresholds. Drugs that shortened or decreased the action of the catecholamines decreased ICSS rates and increased ICSS thresholds. Falling into the first category are the catecholamine releasing agents such as the amphetamines, the catecholamine reuptake inhibitors such as cocaine, and the direct catecholamine receptor agonists such as apomorphine. Into the latter category fall
the catecholamine neurotransmitters such as 6-hydroxydopamine, the catecholamine synthesis inhibitors such as alpha-methyl-para-tyrosine and the catecholamine receptor antagonists such as chlorpromazine.

The above evidence for the involvement of the catecholamines in the neural substrates of reward is rendered less convincing by the fact that it is based solely on increases or decreases in ICSS rates or current thresholds. A drug-induced decrease of ICSS does not necessarily mean that the reward value of brain stimulation has been reduced by the drug, that is, that the drug has produced a motivational or reward deficit. Instead, the animal may have ceased to respond because of a drug-produced general malaise, motor deficit, sensory deficit or an attentional deficit, that is, what has become known as a performance deficit. Similarly an increase in ICSS rates need not be interpreted as indicating that the drug treatment increased the rewarding value of the brain stimulation. Perhaps the increase in ICSS reflects a general activational effect on all behavior. Thus the drug-induced decreases and increases in
ICSS rates could be the result of an alteration of the animal's ability to perform tasks such as lever pressing rather than an alteration of the rewarding magnitude of the brain stimulation.

The problem of whether drugs cause performance deficits or reward deficits is best illustrated by the following example. Wise and Stein (1969) reported that disulfiram, an inhibitor of dopamine-β hydroxylase, markedly reduced lateral hypothalamic ICSS rates. This attenuation could be reversed by the intraventricular administration of l-norepinephrine. Wise and Stein (1969) interpreted these findings as indicating that NA systems were mediating the observed ICSS. Another interpretation of these data is that the disulfiram caused a performance deficit that reduced the animals' ability to maintain responding. The fact that l-norepinephrine temporarily reinitiated responding may merely indicate that the l-norepinephrine temporarily reversed the disulfiram induced performance deficit. This latter interpretation is supported by Roll (1970). She too observed that disulfiram reduced ICSS rates, but also noted that
the animals appeared lethargic. When these animals were aroused by handling, they lever-pressed at normal rates only to gradually fall asleep once again (Roll, 1970). While the data presented by Roll (1970) are by themselves inconclusive since she based her observations on only a few animals, they do identify the need to separate nonspecific performance deficits from reward or motivational deficits.

A similar problem arises when one attempts to attribute decreases in reward to a reduction in DA synaptic function. Low levels of brain dopamine are associated with Parkinson's disease (Hornykiewicz, 1965) and profound sensory neglect (Ungerstedt, 1971b; Marshall, Richardson; and Teitelbaum, 1974). Even normally important incentive stimuli do not appear to have their usual activational effects. Indeed, other investigators have attributed the reduction of ICSS rates by 6-hydroxydopamine, DA receptor blockers and catecholaminergic synthesis inhibitors to interference with volitional movement (Rolls, Rolls, Kelly, Shaw, Wood, and Dale, 1974) or to reduced ability to initiate and maintain motor
behavior (Phillips, Brooke, and Fibiger, 1975; Fibiger, Carter, and Phillips, 1976). Similar data have been interpreted by Fouriezos and Wise (1976) to result from a reduction in the reward value of the stimulation. These investigators observed extinction-like patterns of responding in ICSS tests after pimozide pretreatment. The response patterns of the pimozide animals were similar to the response patterns of drug-free animals following reduction of the stimulation currents to subthreshold values. However, these data are open to other interpretations. The fact that the animals responded initially at normal rates when put in the ICSS test chamber could be accounted for by the arousing effects of the handling and the powerful incentive cues of the test chamber being sufficient to temporarily overcome the debilitating effects of the pimozide. It is commonly known that the Parkinsonian patient sometimes responds to highly motivating stimuli by exhibiting brief periods of controlled volitional movement. Edmonds and Gallistel (1977) have also developed a paradigm that seems to separate reward and performance effects. These authors found evidence supporting
the notion of both reward and performance deficits in rats pretreated with alpha-methyl-para-tyrosine. However, their test drug is known to have non-specific side effects (Olds, 1976; Edmonds and Gallistel, 1977) and thus their results are not as helpful as they might have been had they used a more selective drug such as pimozide. It is interesting to note, that NA receptor blockers such as phenoxybenzamine do not produce the extinction-like patterns of responding seen with DA receptor blockers but instead produce a uniformly suppressed pattern of responding indicative of a true performance deficit (Fouriezos, 1976; Note #1). The studies of Fouriezos and Wise (1976) and Edmonds and Gallistel (1977) have by far come the closest in adequately dealing with the reward deficit-performance deficit issue.

In summary, reduced DA function has been linked to inability to initiate and maintain voluntary behavior, to a reduction in the activational effects of sensory events and to deficits in affect. Perhaps dopamine is required for the normal expression of all of these functions. In any event, the validity of the catecholamine hypothesis of
ICSS will not be settled on the basis of the pharmacological evidence alone.

Anatomy of ICSS

While the catecholamines were implicated in ICSS primarily on the basis of pharmacological data, several researchers noted that ICSS sites correlated with those areas of the brain high in catecholamine content (Stein, 1967; Dreese, 1966a; Crow, 1972). The description of the ascending NA and DA systems in rat brain by Ungerstedt (1971) and Lindvall and Bjorklund (1974) prompted German and Bowden (1974) to replot existing ICSS maps onto these newly described catecholamine maps. This analysis revealed a substantial overlap between ICSS sites and the loci of the catecholamine systems, that is, the cell bodies of origin, efferent fibers and synaptic terminals.

ICSS has been obtained repeatedly from the region of the substantia nigra and interpeduncular nucleus (Dreese, 1966a; Olds et al., 1960; Olds and Olds, 1963; Routtenberg and Malsbury, 1969; Huang and Routtenberg, 1971; Crow, 1972; Prado-Alcala, Kent, and Reid, 1975) wherein are located the A8, A9 and A10 cell groups that give
rise to the nigrostriatal, mesolimbic and mesocortical DA systems (Ungerstedt, 1971; Lindvall and Bjorklund, 1974). In addition, ICSS has been demonstrated along the fiber bundles of the DA systems as they run through the medial forebrain bundle and medial to or within the tip of the internal capsule (Olds et al., 1960; Olds and Olds, 1963; Routtenberg, 1971; Prado-Alcala et al., 1975). ICSS has also been observed from the caudate nucleus (Olds et al., 1960; Olds and Olds, 1963; Routtenberg, 1971; Phillips, Carter, and Fibiger, 1976), the nucleus accumbens (Phillips et al., 1975; Routtenberg, 1971; Olds and Olds, 1963) and the frontal, cingulate and entorhinal cortices (Routtenberg, 1971; Routtenberg and Sloan, 1972; Collier and Routtenberg, 1977; Olds and Olds, 1963). All of the above areas receive substantial projections from the midbrain DA cell groups.

In summary, ICSS appears to be reliably obtained from all levels of the three major DA systems. The same relation does not so clearly characterize the NA systems.
The ventral NA system (Ungerstedt, 1971) has its origin in the medullary-pontine A1, A2, A5-A7 cell groups. Attempts to obtain ICSS from the A1, A2 and A5 cell groups with (Anlezark, Arbuthnott, Christie, Crow, and Spear, 1973) and without behavioral shaping (Clavier and Routtenberg, 1974) have not been successful. Ritter and Stein (1974) have attributed ICSS from caudal midbrain sites to activation of the A6 and A7 components of the ventral NA bundle. Despite this claim, it is generally thought that the ventral NA bundle does not participate in ICSS (German and Bowden, 1974; Clavier and Routtenberg, 1974; Crow, 1976).

The evidence that has been interpreted to implicate the dorsal tegmental NA system (dorsal NA bundle of Ungerstedt, 1971) in ICSS is substantial, but controversial. The dorsal tegmental NA system has its origin in the NA A6 cell group, an area that corresponds to the locus coeruless (Ungerstedt, 1971; Lindvall and Bjorklund, 1974). ICSS has been reported from the locus coeruless (Crow, Spear, and Arbuthnott, 1972; Ritter and Stein, 1973; Micco, 1974; Ellman,
Ackermann, Farber, Mattiace, and Steiner, 1974), from the midbrain trajectory of the dorsal tegmental NA bundle (Crow, 1972; Crow et al., 1972; Ritter and Stein, 1973) as well as from various terminal areas of the dorsal tegmental bundle such as the hippocampus (Ursin, Ursin, and Olds, 1966), the amygdala (Wurtz and Olds, 1963), and the cingulate cortex (Olds and Olds, 1963; Routtenberg, 1971). In addition to the mapping studies a number of investigators have shown that ICSS increases the release or turnover of catecholamines as determined from brain perfusates (Stein and Wise, 1969; Holloway, 1975), reduction of terminal area fluorescence (Arbuthnott, Crow, Fuxe, Olson and Ungerstedt, 1970; Arbuthnott, Fuxe, and Ungerstedt, 1971) and cortical assay techniques (Anlezárk, Walter, Arbuthnott, Crow, and Ecclestone, 1975).

From the mapping studies reviewed above it would appear that ICSS is associated with all three midbrain DA systems and the dorsal tegmental NA system. The ventral NA system does not appear to support ICSS (but see Ritter and Stein, 1974).
The anatomical data relating ICSS to the catecholamine pathways are problematic since they were derived from the pharmacological data discussed earlier. The approach taken (e.g. German and Bowden, 1974; Crow, 1976) has been to use the findings of pharmacological studies to infer the neurochemical (e.g. norepinephrine) basis of ICSS and then look for supporting biochemical or anatomical evidence indicating that NA systems do occupy those regions of the brain from which ICSS can be obtained. This line of reasoning is weakened by the aforementioned ambiguities in interpreting the pharmacological data. Moreover, not only do the catecholamine systems occupy a small portion of overall brain circuitry, they are characterized by their widespread and diffuse projections (Ungerstedt, 1971; Lindvall and Bjorklund, 1974). The striking correlation between ICSS sites and the loci of the catecholamine system is not surprising when one considers the diffuseness of these systems.

In addition, none of the investigators who have attributed ICSS to activation of particular catecholamine systems have used the
fluorescent histochemical techniques that are necessary for the visualization of catecholamines. Instead, the procedure has been (Crow, 1972; Crow et al., 1972; German and Bowden, 1974; Ritter and Stein, 1974; Crow, 1976) to transpose existing ICSS maps (Olds and Olds, 1963; Routtenberg and Halsbury, 1969; Routtenberg, 1971) onto the catecholamine maps of Ungerstedt, (1971) and Lindvall and Bjorklund (1974). This type of analysis is problematic for several reasons.

First, the ICSS maps have been based on the results of studies in which the ICSS current levels were high and the histological data were of poor quality or lacking in detail. For example, Olds and Olds (1963) used sinusoidal currents of 50 μA with electrode sites spaced at 1.0 mm intervals in mapping ICSS sites in the diencephalon and rostral midbrain. The use of such high stimulating currents which may have an effective spread of a 1.0 mm (Olds et al., 1960) makes it difficult to specify the neural systems that mediate the ICSS. The lack of finer steps between electrode placements adds to the problem. Atrens (1973) has also criticized the above study
on the basis of the stimulation currents as well as the small number of electrode sites tested in each area. Routtenberg and colleagues (Routtenberg and Malsbury, 1969; Routtenberg, 1971; Huang and Routtenberg, 1971; Routtenberg and Sloan, 1972; Clavier and Routtenberg, 1974) have avoided some of the problems of other mapping studies by employing low ICSS currents in combination with small diameter stimulating electrodes. However, Routtenberg and associates do not shape or train their animals to lever press for brain stimulation as is customary in most ICSS laboratories. Instead, the animals are placed in the test chamber for fifteen minutes per day and are left to acquire ICSS without experimenter assistance. These procedures, though allowing comparisons between positive sites, make the interpretation of negative data difficult. That is, ICSS may be obtainable from some sites only if the animals are shaped to self-stimulate. It appears that shaping may be necessary in order to obtain ICSS at sites in the dorsal pontine tegmentum (Crow, 1976), the caudate nucleus (Phillips et al., 1976) and the medulla (Carter and Phillips,
1975). In addition, the use of a single, low current intensity may have prevented Routtenberg and associates from obtaining ICSS from regions in which the systems underlying ICSS were diffusely arranged. In such a region, currents of more than 25 ua may be necessary to reach ICSS threshold levels. In other regions this current level may be too high and thus stimulation may be aversive.

The relative imprecision and ambiguities of the early ICSS mapping studies make it difficult to relate them to such diffuse neural systems as the catecholamine systems.

A second problem is that the catecholamine maps as described by Ungerstedt (1971) and Lindvall and Bjorklund (1974) are highly schematic and of purposefully exaggerated prominence with respect to the rest of the brain. Lindvall and Bjorklund (1974, pg. 33), made the following statement, "to a very large extent, the adrenergic pathways follow - along or reciprocal to - well established non-adrenergic fibre tracts. Thus a number of fiber systems, well known from the classical neuroanatomy, also carry adrenergic axons...". These observations did not prevent some
investigators from assuming that the catecholamine systems occupied a significant portion of the stimulation field beneath their ICSS electrodes. For example, Ritter and Stein (1974) attributed ICSS at caudal mesencephalic sites to activation of the ventral NA bundle as described by Ungerstedt (1971) and Lindvall and Bjorklund (1974). The ventral NA bundle fibers which are themselves loosely arranged, occupy the ventral portion of the central tegmental tract which consists of both ascending and descending non-catecholamine fibers (Lindvall and Bjorklund, 1974). The diffuse nature of the ventral NA bundle suggests that Ritter and Stein (1974) were unjustified in claiming that it was this system that was supporting ICSS.

Third, the demonstrations of increased catecholamine turnover following ICSS (Stein and Wise, 1969; Holloway, 1975; Arbuthnott et al., 1971; Anlezark et al., 1975) in themselves, do not causally link the catecholamines to ICSS. Rather, these data merely demonstrate that catecholamine fibers occupy some portion of the stimulation field. It is likely that stimulation at a variety
of ICSS sites would result in increased turnover of GABA, histamine, acetylcholine and numerous other putative neurotransmitters since these compounds are located in brain regions that support ICSS.

Finally, it is now questionable whether any NA systems support ICSS at all, since there is no longer certainty as to whether the dorsal tegmental NA system supports ICSS. While ICSS has been reported from the region of the locus coeruleus (Crow et al., 1972; Ritter and Stein, 1973; Segal and Bloom, 1976) the nucleus of origin of the dorsal tegmental NA system, both Amaral and Routtenberg (1975) and Simon, LeMoal, and Cardo (1975) failed to obtain ICSS from electrodes localized to the locus coeruleus. As pointed out by Amaral and Routtenberg (1975) most of the representative locus coeruleus electrode placements in the studies of Crow et al. (1972) and Ritter and Stein (1973) lay outside the locus coeruleus. Also questioning the view that the locus coeruleus supports ICSS are the findings by Clavier, Phillips, and Fibiger (1976) and Corbett, Skelton, and Wise (1977) that lesions of the dorsal tegmental NA bundle fail to disrupt ICSS from the
region of the locus coeruleus. Cooper and Breese (1976) were also unable to disrupt ICSS from the region of the locus coeruleus despite extensive whole brain depletions of norepinephrine. While these data would seem to virtually preclude a role for the dorsal tegmental NA system in ICSS, they do not. The investigators (Amaral and Routtenberg, 1975; Simon et al., 1975) who failed to obtain ICSS with electrodes in the locus coeruleus did not use behavioral shaping techniques, a method that may be necessary to obtain ICSS from certain brain regions (Crow, 1976; Phillips et al., 1976; Carter and Phillips, 1975). It is possible that ICSS can be obtained from the locus coeruleus only after careful and extensive behavioral shaping. The failure of dorsal tegmental NA bundle lesions (Clavien et al., 1976; Corbett et al., 1977) or whole brain depletions of norepinephrine (Cooper and Breese, 1976) to affect ICSS from the region of the locus coeruleus may reflect the multiplicity or redundancy of the reward circuitry (Valenstein, 1966; Lorens, 1976).

In summary then, it has to be concluded that the purported correlation between the catecholamine
systems and ICSS loci is at best approximate. As discussed above, many of the original ICSS mapping studies were imprecise with regard both to stimulation parameters and to histological data. In addition, there has been a widespread failure to appreciate the diffuseness of the catecholamine systems. Finally, there is controversy regarding the reliability of particular findings. These general criticisms of the anatomical data together with the difficulties of interpreting the pharmacological data suggest that the catecholamine hypothesis of ICSS as expressed by German and Bowden (1974) and Crow (1976) is no longer convincing at least on the basis of existing data.

The present thesis reflects an anatomical approach to the question of whether ICSS is the result of the activation of one or more catecholamine systems. Mapping the catecholamine systems for ICSS more precisely and systematically than has been done previously (Ritter and Stein, 1973; Crow, 1972; Crow et al., 1972) might provide information that would assist in the interpretation of the pharmacological data. In addition, present pharmacological methods do not distinguish between
neural systems which have the same neurotransmitter. That is, a DA receptor blocker does not distinguish between mesolimbic, mesocortical or nigrostriatal DA systems. By carefully placing the stimulating electrodes and utilizing low current intensities it should be possible to preferentially stimulate some of the DA systems.

Since the approach in this thesis is to correlate changes in ICSS rate and thresholds with the loci of the catecholamine systems it was decided to use fluorescent histochemical methods to directly verify electrode placements. The shortcomings of trying to transpose ICSS electrode placements onto the catecholamine maps of others are obvious. If catecholamine systems do mediate ICSS, then ICSS should be restricted to the boundaries of these systems. Also, the magnitude or vigour of ICSS might be expected to be proportional to the density of catecholamine elements beneath the electrode tip. Thus ICSS rates might be higher and current thresholds lower in areas where the catecholamine density is high, while ICSS rates might be lower and current thresholds higher in areas where the
catecholamine density is low. Even if a high correlation were always found between ICSS sites and catecholamine systems it would be difficult to interpret. Such a correlation would not be necessarily meaningful since the catecholamine systems are so diffuse. In order to circumvent this problem, a moveable, rather than a fixed stimulating electrode was employed. This electrode (Wise, 1976) following implantation, could be lowered in steps of 125 μm or 250 μm for a total travel of 2.0 mm. Multiple stimulation sites could be tested in the same animal and ICSS rates and thresholds at each stimulation site correlated with the relative catecholamine density along the electrode penetration. While there is not yet any precise means for determining the distance that current spreads from an electrode, the best estimates at present (Ranck, 1975) for the currents used in this thesis are in the range of 100-500 μm. However, since the stimulating electrodes used in this investigation are not fixed, it is possible to stimulate different neural elements by lowering the electrode. The use of a moveable electrode allowed within-subject comparisons of ICSS response
rates and current thresholds with electrode location, a distinct advantage over fixed electrode mapping studies where comparisons necessarily have to be made between subjects. These latter comparisons are not as precise since differences in electrode configurations, emotionality, response topography, ... etc. could all contribute to differences in response rates and current thresholds.

The area mapped for ICSS extended from the caudal level of the locus coeruleus to the mid-hypothalamic level. In view of the considerable controversy still surrounding the role of the locus coeruleus in ICSS (Crow, 1976; Hall et al., 1977) it was decided that the locus coeruleus area should be remapped for ICSS with behavioral shaping techniques and the greater anatomical precision offered by the moveable electrode to finally determine whether or not ICSS can be obtained from the locus coeruleus itself. Of particular interest in the present study was the examination of the suggested correlation between ICSS sites and the midbrain DA cell groups (Dreese, 1966a; Crow, 1972, 1976; German and
Bowden, 1974). These DA cell groups have been regarded as being equally capable of supporting ICSS (Crow, 1973, 1976; German and Bowden; 1974). This assumption is somewhat surprising considering the marked anatomical differences between the nigrostriatal, mesolimbic and mesocortical DA systems. These anatomical differences also seem to reflect marked functional differences since dysfunction of the nigrostriatal DA system seems associated with movement disorders such as Parkinson's disease (Hornykiewicz, 1966) whereas the mesolimbic and mesocortical DA systems seem concerned with affective tone. Dysfunction of these latter systems has been hypothesized to play a role in schizophrenia (Snyder, Banerjee, Yamamura, and Greenberg, 1974).
METHOD

Subjects:

One hundred and ten male Sprague-Dawley rats obtained from Canadian Breeding Farms Ltd. and weighing 250-450 g at the time of surgery were used in the present experiment. Surgery was performed under sodium pentobarbital anesthesia (60 mg/kg, i.p.) in combination with the muscarinic antagonist drug atropine sulfate (.3 mg/kg, s.c.). In addition, all animals received 60,000 i.u. (i.m.) of Penicillin G following surgery. Each rat was implanted with a monopolar moveable electrode. The electrode was constructed of 254 um diameter stainless steel wire concentrically soldered into a male Amphenol connector which had been threaded externally with a 2-56 thread die. The electrodes were insulated with Formvar except at the electrode tip which was sharpened to a conical point. The threads of the Amphenol pin were liberally covered with stopcock grease (Dow Corning) prior to screwing the electrode into a threaded nylon receptacle 10 mm in length and 4 mm in diameter. Additional stopcock grease was applied to the base of the receptacle until 2-3 mm of the
protruding electrode shaft were insulated. When the electrode assembly was implanted the stopcock grease formed a seal between the skull and the nylon receptacle thus preventing: (1) dental cement from encroaching upon the electrode shaft; and (2) cerebrospinal fluid from seeping up the nylon receptacle to the uninsulated threads of the Amphenol pin and shorting the stimulating electrode to the skull screw ground. Once implanted, the electrode could be lowered in steps of approximately 125 μm or 250 μm by grasping the 2-56 threaded Amphenol pin with a pin vice and rotating it clockwise ¼ or ½ revolution. A thin line of nail-polish painted on the Amphenol pin served as a guide for determining the degree of rotation of the pin vice. The maximum ventral travel of the electrode was 2.0 mm. This distance could be achieved in eight 250 μm steps or sixteen 125 μm steps. A skull screw served as the indifferent electrode.

The area mapped for ICSS extended from the caudal level of the locus coerulesus to the mid-hypothalamic level. This area corresponds approximately in the deGroot plane to the following
coordinates derived from Bregma: anterior-posterior: -0.4 mm to -8.6 mm; medial-lateral: 0.0 mm to 2.5 mm; and 5.0 mm to 9.0 mm ventral from the dural surface.

Each animal was allowed at least 3 days to recover from surgery before testing for ICSS was initiated.

Apparatus:

Self-stimulation testing was conducted in standard operant test chambers equipped with a Gerbrands lever (Part #G6312) that when depressed, triggered a 500 msec train of 60 Hz, sine-wave, stimulation.

Procedure:

The procedure consisted of two phases. During Phase 1 the animals were screened for ICSS and any animals who self-stimulated were tested until responding was reliable at which time the animals were switched to Phase 2. Phase 2 was a standard rate intensity paradigm. Once an animal was switched to Phase 2 it was never returned to Phase 1, unless of course the animal failed to self-stimulate on its first day of Phase 2, in which case it was returned to Phase 1 for a few
additional days of training. Following completion of testing at the first ICSS site of Phase 2, the electrode was lowered and rate-intensity testing was begun at the second electrode site. The electrode was lowered in this manner until the animal no longer self-stimulated or the electrode had been lowered to its full extent. At this time the animals were sacrificed.

Phase 1 consisted of screening the animals for ICSS in daily 15 min. test sessions at current intensities up to but not exceeding 50 μA. The duration of screening at the first electrode site in a given animal was determined by the behavior elicited by the stimulation: (1) if low current stimulation (e.g. 20 μA) was clearly aversive, the electrode was immediately lowered (250 μm) and screening resumed at the next site 24 hr. later; (2) if the stimulation appeared neutral at currents up to 50 μA for two consecutive days, the electrode was lowered to the next site and screening resumed 24 hr. later; (3) in the event that the stimulation appeared to have possible rewarding properties as indicated by increased locomotion, approach to the lever,
gnawing, etc., screening was maintained until the animal began to self-stimulate or until further shaping seemed pointless (in the latter case, the electrode was lowered to the next site) or (4) when ICSS was obtained, the animals were allowed 2-5 days of responding in 15-30 min test sessions until responding was reliable, before being switched to Phase 2.

Phase 2 consisted of the collection of rate-intensity data from each electrode site tested in each animal. Testing at each electrode site was of 3 days duration (this was later increased to 5 days). The daily test sessions were 35 min. in duration. A descending series of up to seven different current intensities was used in each 35 min. test session. The range of current intensities selected for each animal had been determined during Phase 1. At the end of each 5 min. segment of the rate-intensity test session the current was reduced by 3.0 μA (1.0 μA at lateral hypothalamic sites). The first current intensity that failed to maintain a response rate of 10 responses per min. was considered the threshold current for ICSS. The same stimulation
currents were used on each of the 3 test days. Following the collection of the rate-intensity data on the third test day, the electrode was lowered to the next electrode site; rate-intensity data were collected for 3 test days; the electrode was lowered to the next site; ... etc. Testing was terminated when the animal no longer self-stimulated or when the electrode had been lowered to its maximal travel of 2.0 mm. When the ICSS at a particular electrode site was of special interest (e.g. low threshold ICSS) the electrode was not lowered at the end of the 3 days of rate-intensity data collection. Instead, the animal was prepared for histological examination. If a marked change in ICSS current threshold or rate occurred as the result of the electrode being lowered through some boundary region, the rate-intensity data were collected for this site and then the animal was sacrificed.

In the early stages of this study it was observed that lowering the electrode 125 μm generally did not result in substantial changes in either ICSS thresholds or rates. Thus the remaining 79 rats had their electrodes lowered.
in steps of 250 μm. Since the total mapping time was reduced by lowering the electrodes in steps of 250 μm, the rate-intensity test days at each electrode site were increased from three days to five days. These procedural changes are included with each animal's rate intensity and threshold data.

**Histology:**

At the conclusion of testing, 67 of the animals were anesthetized with sodium pentobarbital (60 mg/kg) and perfused with physiological saline, followed by 10% formalin. The brains were removed and stored for a minimum of 3 days in a 10% formalin solution. Frozen, 40 μm brain sections were cut in the deGroot plane and stained with thionin for examination and subsequent reconstruction of electrode placements.

The remaining 43 animals were treated with the glyoxylic acid method of Battenberg and Bloom (1976) for the demonstration of catecholamines. The method was as follows: 7–21 days after the last lowering of their electrode, the animals were anesthetized with chloral hydrate (400 mg/kg,
and perfused transcardially with an ice-cold (2-4°C) phosphate buffered Ringer's solution containing 0.5% paraformaldehyde and 2% glyoxylic acid. A total of 250-400 ml of perfusate was rapidly infused (90-120 sec.) in each animal by using compressed air as a propellant. Following the perfusion, the brains were rapidly removed, blocked and frozen on dry ice before being placed in a cryostat. Serial 20 μm sections were cut in the coronal plane and thawed onto pre-chilled glass slides which were then quickly immersed in a 2% glyoxylic acid bath solution at 2-4°C for 2-5 min. The slides were removed from the bath solution, dried in a warm air stream until just visibly dry and then incubated in a covered glass container at 100°C for 10 min. The sections were then examined with a Leitz fluorescence microscope using epi-illumination from a 200 W mercury lamp source in combination with a 355-425 nm excitation filter and a 460 nm barrier filter. Photomicrographs were taken using Kodak Tri-X film which was processed normally in Microdol-X developer.
RESULTS

A total of 676 electrode sites were tested for ICSS in 110 rats. Two hundred and sixty of these electrode sites (38.5%) supported ICSS while the remaining 416 sites (61.5%) did not support ICSS. The results are described in relation to the NA and DA systems.

ICSS in Relation to the Ascending NA Systems

Twelve animals had electrodes that had passed through or were adjacent to the locus coeruleus. These electrode penetrations as well as all other electrode placements in the region of the locus coeruleus are represented in Figure 1 (cerebellar placements are not included). Since the moveable electrodes were lowered in steps of 125 µm or 250 µm, several sites were tested for ICSS along the dorso-ventral extent of the locus coeruleus. Figures 2 and 3 are composite fluorescent micrographs of the locus coeruleus electrode placements of animals #49 and #106. Figure 5 shows adjacent brain sections taken from the same animals but stained with thionin. Note that #49's electrode has passed along the medial edge of the locus coeruleus while #106's electrode is in the dorsal aspect of the locus coeruleus.
Figure 1. Horizontal reconstruction of electrode penetrations in the region of the locus coeruleus. Abbreviations: DTG = dorsal tegmental nucleus of Gudden; IV = fourth ventricle; LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve.
Figure 2. Composite fluorescence micrograph of the electrode tract of animal #49. Electrode tip is indicated by an arrow. Indicator bar = 1.0 mm. Abbreviations: LC = locus coeruleus.
Figure 3. Composite fluorescence micrograph of the electrode tract of animal #106. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: LC = locus coeruleus; PCS = superior cerebellar peduncle.
Figure 4. Composite fluorescence micrograph of the electrode tract of animal #51. Electrode tip is indicated by an arrow. Indicator bar = 1.0 mm. Abbreviations: DTB = dorsal tegmental noradrenergic bundle; PCS = superior cerebellar peduncle.
Figure 5. Thionin stained sections of the electrode tracts of animals 49, 106, 34, 37, 45, 46, 48, and 51. Electrode tip is indicated by an arrow.

Abbreviations: LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve; Mot. V = motor nucleus of the trigeminal nerve; PCS = superior cerebellar peduncle; RPO = nucleus reticularis pontis oralis.
Six different electrode sites were tested for ICSS in rat #49 making a total of 21 test sessions (one per day). Rat #106 was tested for ICSS at only one electrode site for a duration of 19 test sessions. Neither animal displayed ICSS but both exhibited vigorous gnawing upon delivery of priming stimulation. This gnawing undoubtedly was the result of current spread to the nearby mesencephalic nucleus of the trigeminal nerve (Mes. V).

The brains of six additional animals were prepared with the glyoxylic acid method and were found to have electrode placements that traversed or terminated in one of the NA fiber bundles running through the pons and caudal midbrain. Five of these placements are schematically illustrated in relation to the observed catecholamine fluorescence for each individual animal in Figure 6. The corresponding thionin sections of these animals are shown in Figure 5. The ICSS thresholds and rate-intensity scores at each electrode site for four of the animals (#'s 34, 45, 46, and 48) are shown in Figures 7, 8, 9, and 10. The fifth animal (#37) did not self-stimulate. The remaining animal's
Figure 6. Schematic representation of the electrode tracts of animals 34, 37, 45, 46, and 48. The electrode penetration is shown in the left of each section and the observed catecholamine fluorescence is shown in the right of each section. Filled circles indicate self-stimulation sites. The number of the self-stimulation site (e.g. first, second, etc.) is shown by arrows. Animal #37 did not self-stimulate. The most dorsal and ventral electrode sites in this animal are indicated by open circles. Abbreviations: CTT = central tegmental tract; DTB = dorsal tegmental noradrenergic bundle; LC = locus coeruleus.
Figures 7-11. Self-stimulation current threshold and rate-intensity data at each self-
stimulation site for animals 34, 45, 46, 48, and 51. The current thresholds at each electrode site are shown in the upper left panel. The response rates at each current intensity (rate-intensity) at each of the first three self-stimulation sites are shown in the upper right panel, the fourth, fifth, and sixth self-stimulation sites in the lower left panel, and the remaining self-stimulation sites in the lower right panel. Filled circles, triangles, and squares indicate the first, second, and third electrode sites respectively. This sequence is repeated for each of the two remaining rate-intensity panels. The interval between each self-stimulation site was 250 μm.
electrode placement is depicted in a composite fluorescence montage (#51, Figure 4) and the adjacent thionin stained section is shown in Figure 5. Threshold and rate-intensity data for this animal can be found in Figure 11.

The density of NA fluorescence within a 500 um diameter sphere was rated on a three point scale (Appendix II) for each electrode site supporting ICSS from the above animals (#'s 34, 45, 46, 48, and 51). There was no correlation between the density of NA neural elements and the ICSS current thresholds at these electrode sites (Spearman \( \rho = .03 \), not significant).

Forty-two of seventy-nine (53.2\%) electrode placements in or adjacent to the dorsal raphe nucleus were found positive for ICSS. High rates of ICSS with low current thresholds were obtained from rostral portions of this nucleus (e.g., #5, Figures 12 and 13) whereas low rate, high current threshold ICSS characterized caudal portions of the dorsal raphe nucleus (e.g., #9, Figures 12 and 14).

The distribution of ICSS loci within the superior cerebellar peduncle (PCS) was not uniform. High rate,
Figure 12. Thionin stained sections of the electrode tracts of animals 5, 9, 33, 35, 47, 123, 124, and 140. Electrode tip is indicated by an arrow. Abbreviations: DMH = dorsomedial hypothalamic nucleus; DPCS = decussation of the superior cerebellar peduncle; Mes. V = mesencephalic nucleus of the trigeminal nerve; Mot. V = motor nucleus of the trigeminal nerve; MR = median raphe nucleus; PCS = superior cerebellar peduncle.
Figures 13-16. Self-stimulation current threshold and rate-intensity data of animals 5, 9, 47, and 35. The interval between each self-stimulation site was 125 μm in animals 5 and 9 and 250 μm in animals 35 and 47. The data are illustrated as described for Figures 7-11.
low threshold ICSS was obtained from medial portions of the PCS as it merged with the pontine tegmental gray, these latter two areas being intersected by the Mes. V (e.g., #47, Figures 12 and 15). Stimulation of medial portions of the PCS tended to yield low rate and medium to high (35 μa - 50 μa) current threshold ICSS. Far-lateral portions of the PCS generally did not support ICSS (e.g., #35, Figure 12).

Self-stimulation was obtained adjacent to the mid-rostral aspect of the Mes. V (Figure 1) as well as dorso-medial to and within the medial portion of the motor nucleus of the trigeminal nerve (Mot. V). Examples of these Mot. V placements are shown in Figure 12 (#35) and Figure 5 (#34). The ICSS threshold and rate-intensity data for #35 are illustrated in Figure 16.

Among those areas found negative for ICSS, in addition to those mentioned above, were the facial nerve, the dorsal tegmental nuclei of Gudden, the dorsal half of the periaqueductal gray, the inferior peduncle and the ventromedial layers of the cerebellum. Interestingly, one electrode placement
that was localized to the median raphe nucleus, failed to support ICSS (#140, Figure 12). Medial hypothalamic placements in or near the paraventricular and dorsomedial hypothalamic nuclei did not support ICSS. Fluorescent, composite photographs of such electrode placements are shown in Figure 17 (#123) and Figure 18 (#124). The corresponding thionin stained sections from these two animals (#123 and #124) are seen in Figure 12. Additional examples of the above placements can be found in Appendix I (dorsal raphe, Figure 43; PCS, Mes. V and Mot. V, Figures 50, 57; peri-coeruleus, Figures 63 and 65).

**ICSS in Relation to the Ascending DA Systems**

One hundred and twenty-one of 252 electrode sites (48%) within the area extending from the substantia nigra to the mid-hypothalamic level, were found to support ICSS. Self-stimulation was more readily obtained from DA sites than from NA sites ($\chi^2 = 14.73, p < .001$). Summary maps of the DA electrode placements are illustrated in Figures 19, 20, and 21. The ICSS current thresholds and rates shown in each cell of these figures are median scores that were determined from the median ICSS
Figure 17. Composite fluorescence micrograph of the electrode tract of animal 123. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: PVH = paraventricular nucleus of the hypothalamus.
Figure 18. Composite fluorescence micrograph of the electrode tract of animal 124. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: DMH = dorsomedial nucleus of the hypothalamus; FX = fornix.
Figure 19. Summary diagram of self-stimulation current thresholds (left plate) and rates (right plate) at the mid-hypothalamic level. The number in the left hand corner of each brain section indicates the anterior-posterior distance of the brain section from bregma (Pellegrino and Cushman, 1967). The numbers in the right hand corner of each brain section identify the subjects that were used in compiling each plate. The numbers in each cell of the left hand plates are median self-stimulation current thresholds. Filled circles indicate self-stimulation rates greater than 80 responses per minute, filled squares 50-79 responses per minute, filled triangles 20-49 responses per minute, and filled diamonds 5-19 responses per minute. Open cells indicate that the area within the cell was not tested for self-stimulation. A minus sign indicates that the majority of placements within the cell did not support self-stimulation. Abbreviations: DMH = dorsomedial nucleus of the hypothalamus; FX = fornix, lC = internal capsule; MFB = medial
forebrain bundle; ML = medial lemniscus; OT = optic tract; PVH = paraventricular hypothalamic nucleus; VMH = ventromedial hypothalamic nucleus; ZI = zona incerta.
Figure 20. Summary diagram of self-stimulation thresholds and rates at the posterior hypothalamic level. The data are illustrated as described for Figure 19. Abbreviations: FX = fornix; FR = fasciculus retroflexus; IC = internal capsule; MFB = medial forebrain bundle; ML = medial lemniscus; MT = mamillothalamic tract; VTN = ventral tegmental nucleus of Tsai; ZI = zona incerta.
Figure 21. Summary diagram of self-stimulation thresholds and rates at the level of the midbrain dopamine cell groups. The data are illustrated as described for Figure 19. Abbreviations:

1P = interpeduncular nucleus; ML = medial lemniscus;
RN = red nucleus; SN = substantia nigra;
VTN = ventral tegmental nucleus of Tsai.
current thresholds and rates of individual animals with electrodes that entered a particular cell. In the case of electrode sites not supporting ICSS in a cell in which there were also positive sites, the negative placement was assigned a threshold score of 50 μA and an ICSS rate of 0. Instances of overlapping positive and negative sites were rare and usually occurred at only the most dorsally tested sites. Open cells indicate that the area within the cell was not tested for ICSS. Cells containing a minus sign indicate that all or the majority of sites tested within the cell failed to support ICSS.

At the mid-level of the substantia nigra (Figure 21, Plate 3.8) ICSS was obtained from the ventral portion of the periaqueductal gray at the most dorsally tested electrode sites, ventrally to the dorsal aspect of the interpeduncular nucleus, and laterally to the medial portions of the substantia nigra, pars compacta. The area dorsal to the interpeduncular nucleus and extending laterally to the edge of the medial lemniscus contains the A10 DA cell group (Ungerstedt, 1971; Lindvall and
Bjorklund, 1974; Lindvall, Bjorklund, and Divac, 1978). Fluorescence micrographs of four A10 electrode placements are shown in Figures 22 (#128), 23 (#130), 24 (#101) and 25 (#139). The corresponding thionin stained sections of these electrode placements (#128, #130, #101, and #139) are shown in Figure 26. The threshold and rate-intensity data for these subjects can be found in Figures 27-30.

At more rostral levels (Figure 20, Plate -2.6; Figure 21, Plate -3.2) ICSS was obtained from the region dorsal to the interpeduncular nucleus, the ventral tegmental area of Tsai, and medial portions of the substantia nigra, pars compacta. Examples of electrode placements in these areas are shown in Figures 31 (#100), 32 (#133), and 33 (#102). Thionin sections of these electrode placements are shown in Figure 26. The rate-intensity scores and threshold data for these animals can be seen in Figures 34-36. There was a significant negative correlation (Spearman ρ = -0.70, p < 0.01, two-tailed test) between density of DA neural elements and ICSS thresholds; the lowest ICSS thresholds were in areas rich in DA fibers and cell bodies. The ratings of DA
Figure 22. Composite fluorescence micrograph of the electrode tract of animal 128. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group; 1P = interpeduncular nucleus.
Figure 23. Composite fluorescence micrograph of the electrode tract of animal 130. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A10 = mesolimbic and mesocortical dopamine-containing cell group; lP = interpeduncular nucleus.
Figure 24. Composite fluorescence micrograph of the electrode tract of animal 101. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A10 = mesolimbic and mesocortical dopamine-containing cell group.
Figure 25. Composite fluorescence micrograph of the electrode tract of animal 139. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group.
Figure 26. Thionin stained sections of the electrode tracts of animals 100, 101, 102, 128, 130, 133, and 138. Electrode tip is indicated by an arrow. Abbreviations: IP = interpeduncular nucleus; MB = mamillary bodies; RN = red nucleus; SNC = substantia nigra, pars compacta; SNR = substantia nigra, pars reticulata; VTN = ventral tegmental nucleus of Tsai.
Figures 27-30. Self-stimulation current threshold and rate-intensity data of animals 128, 130, 101, and 139. The interval between each self-stimulation site was 250 μm. The data are illustrated as described for Figures 7-11.
Figure 31. Composite fluorescence micrograph of the electrode tract of animal 100. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell bodies; SNR = substantia nigra, pars reticulata.
Figure 32. Composite fluorescence micrograph of the electrode tract of animal 133. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A10 = mesolimbic and mesocortical dopamine-containing cell group; MB = mamillary bodies.
Figure 33. Composite fluorescence micrograph of the electrode tract of animal 102. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; SNR = substantia nigra, pars reticulata.
Figures 34-36. Self-stimulation current threshold and rate-intensity data of animals 100, 133, and 102. The interval between each self-stimulation site was 250 μm. The data are illustrated as described for Figures 7-11.
density at each of the ICSS sites of the animals used in this analysis (128, 130, 101, 139, 100, 133, 102) can be found in Appendix II.

In the hypothalamus, ICSS was obtained from an area bounded by the fornix in the medial direction and the medial portions of the internal capsule in the lateral direction. In the dorsal-ventral dimension, ICSS was obtained from the subthalamus to the base of the brain (Figure 19, Plates -0.2 and -0.8; Figure 20, Plate -1.8). Thionin stained sections of all hypothalamic electrode placements and associated threshold and rate-intensity data can be found in Appendix I (see Figures 71-90).

Self-stimulation was not obtained from the caudal A10 cell group or the laterally located A8 cell group (Figure 21, Plate -4.6). Composite fluorescence micrographs of such electrode placements are shown in Figure 37 (135, A10) and Figure 38 (131, A8). The corresponding thionin stained sections are illustrated in Figure 39. The mid-lateral portions of the caudal A8 cell group did not support ICSS nor did the lateral portion of the anterior A9 cell group. Fluorescence micrographs of
Figure 37. Composite fluorescence micrograph of the electrode tract of animal 135. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A8 = dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group; LP = interpeduncular nucleus; ML = medial lemniscus.
Figure 38. A composite fluorescence micrograph of the electrode tract of animal 131. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A8 = dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group.
Figure 39. Thionin stained sections of the electrode tracts of animals 103, 117, 131, 132, and 135. Electrode tip is indicated by an arrow.

Abbreviations: CP = cerebral peduncle; IP = interpeduncular nucleus; ML = medial lemniscus; RN = red nucleus; SNC = substantia nigra, pars compacta; SNR = substantia nigra, pars reticulata.
these placements are illustrated in Figures 10-12 (#132, #117, and #103). Thionin-stained sections of these placements are shown in Figure 39.

Other areas found not to support ICSS were the zona reticulata of the substantia nigra, the interpeduncular nucleus, the medial lemniscus, the red nucleus and the periventricular hypothalamic area (see Figures 19, 20, and 21).

Behavioral Observations

Repetitive jaw movements were associated with ICSS from most regions of the dorsal pons. Animals with electrodes in this region often depressed the lever by grasping it in their teeth and rocking it up and down. However, gnawing and ICSS did not always occur together. In any given electrode penetration a number of the stimulated sites yielded vigorous gnawing, but not ICSS. In addition, stimulation at sites in the dorsal pons, lateral to the locus coeruleus elicited licking responses. These licking responses often occurred independently of gnawing and tended not to be locked or bound to the stimulation. The licking was usually directed at the lever or the floor and walls of the test cage. Marked behavioral
Figure 40. Composite fluorescence micrograph of the electrode tract of animal 132. Electrode tip is indicated by an arrow. Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; SNR = substantia nigra, pars reticulata.
Figure 41. Composite fluorescence micrograph of the electrode tract of animal 117. Electrode tip is indicated by an arrow.
Indicator bar = 250 μm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group; SNR = substantia nigra, pars reticulata.
Figure 42. Composite fluorescence micrograph of the electrode tract of animal 103. Electrode tip is indicated by an arrow. Indicator bar = 250 µm. Abbreviations: A9 = nigrostriatal dopamine-containing cell group; A10 = mesolimbic and mesocortical dopamine-containing cell group; SNR = substantia nigra, pars reticulata.
excitation was almost never associated with ICSS in the pons.

A peculiar behavior pattern was sometimes observed with repeated daily stimulation at electrode sites in or adjacent to the locus coeruleus. These animals in their first few shaping sessions would sometimes gnaw on the lever after receiving priming stimulations. This gnawing-pressing response might be maintained for several minutes or occasionally for the duration of the test session (15 min.). On subsequent test days, these animals spent less time engaged in lever-pressing, or failed to approach the lever altogether. Attempts to induce approach by priming stimulations were not successful. Instead, many of these animals now struggled vigorously before being placed into the test box and exhibited increased respiration and evacuation. These behavioral symptoms intensified with further testing and none of these animals were again induced to self-stimulate.

Self-stimulation was rapidly acquired by animals with placements in or near the dorsal raphe nucleus. Reliable ICSS was usually obtained after a
few minutes of behavioral shaping and was characterized by high rates and behavioral excitation similar in form to that exhibited at mid-lateral or peri-fornical hypothalamic ICSS sites. Dopaminergic ICSS sites in and near the A10 cell group yielded high rate ICSS that was characterized not so much by behavioral excitation but by its highly stereotypic form. While all ICSS is somewhat stereotyped, ICSS from the region of A10 appeared to be locked into a particular pattern more so than ICSS at other brain sites. Electrode sites in the lateral hypothalamus yielded high rates of ICSS at low current thresholds. These animals displayed marked behavioral excitation, often biting the lever and displaying ICSS rates in excess of 100 responses per minute. The range of ICSS current intensities tolerated by the lateral hypothalamic animal was often narrow. Thus, for a particular animal the ICSS current threshold might be 18 μA; with an increase in current to 22 μA the animal would press the lever at very high rates and repeatedly jump out of the test chamber. The only other brain regions in which narrow current ranges had to be used were
ICSS sites in and around the dorsal raphe nucleus and parts of the midbrain reticular formation.
DISCUSSION

In keeping with the organization throughout this thesis, the evidence relating ICSS to the NA and DA systems will be discussed separately, beginning with the NA systems.

The view that NA systems mediate pontine tegmental and caudal midbrain ICSS (Crow, 1972, 1976; Crow et al., 1972; Ritter and Stein, 1973, 1974; German and Bowden, 1974) is based on the observation that a number of ICSS sites in these areas are traversed by one or more of the NA systems. Several findings in the present study suggest that NA systems do not underlie ICSS obtained from the dorsal pons and caudal midbrain.

First, it was not possible to obtain ICSS from the locus coeruleus, the nucleus of origin of the dorsal tegmental NA system (Ungerstedt, 1971a; Lindvall and Bjorklund, 1974). This failure would seem somewhat surprising in view of the numerous published reports of ICSS attributed to activation of the locus coeruleus and the dorsal tegmental bundle (Crow, 1972; Crow et al., 1972; Ritter and Stein, 1973; Anlezark et al., 1975; Segal and Bloom,
to obtain ICSS from the locus coeruleus is not without precedent. Both Amaral and Routtenberg (1975) and Simon et al. (1975) were unable to obtain ICSS from electrodes well localized to the locus coeruleus. Because these investigators did not employ behavioral shaping methods their findings have been criticized (Crow, 1976, 1977) and have not had substantial impact. In the present study, testing for ICSS at each electrode site in the region of the locus coeruleus was often continued for five or six test days and sometimes longer (e.g., #49 and #106, 21 and 19 test days respectively). It is thus unlikely that the failure to obtain locus coeruleus ICSS was due to insufficient behavioral shaping. It is possible that damage to the locus coeruleus because of implantation or lowering of the electrode could be extensive enough to preclude ICSS. The locus coeruleus is only 250-350 µm wide, 600 µm in depth at its maximal point and approximately 900 µm long (Swanson, 1976; Amaral and Sinnamon, 1977). Such an explanation seems unlikely, since a number of electrode placements were in close proximity
to the locus coeruleus, and no observable cytological
damage to locus coeruleus neurons was seen (e.g.,
#106, Figure 3; see Appendix I, Figures 63 and 65).
Even in animals in which damage to the locus
coeruleus was evident (#49, Figure 2; #79, Figure 63;
#82, Figure 65), stimulation at the initial electrode
sites (dorsal to the locus coeruleus) would probably
have been sufficient to have spread to the locus
coeruleus before the electrodes were lowered into
and through the locus coeruleus.

It is known that the anterior portion of the
locus coeruleus gives rise to efferents which form the
dorsal tegmental bundle and contribute to the central
tegmental tract and dorsal periventricular system
(Ungerstedt, 1971a; Olson and Fuxe, 1972; Lindvall
and Bjorklund, 1974; Pickel, Segal and Bloom, 1974;
Jones and Moore, 1977). The posterior portion of the
locus coeruleus projects to the spinal cord (Hancock
and Fougerousse, 1976; Satoh, Tohyama, Yamamoto,
Sakamoto, and Shimizu, 1977) and cerebellum
(Ungerstedt, 1971a; Lindvall and Bjorklund, 1974;
Pickel et al., 1974). It might be that these
anatomical differences underlie functional differences
within the locus coeruleus and that stimulation of the anterior but not the posterior pole would lead to ICSS. The data from the present study do not support this suggestion. The distribution of positive and negative ICSS placements within the locus coeruleus region are shown in Figure 1. There is clearly one animal (#66) in which ICSS was obtained from the extreme rostral pole of the locus coeruleus. It is interesting to note that some of the locus coeruleus placements of Crow et al. (1972) and Ritter and Stein (1973) were at the same anterior-posterior level as that of #66, or even somewhat more rostral. On more careful examination of the electrode placement of #66 (Appendix I, Figure 65) it can be seen that the electrode has passed directly through the locus coeruleus which at this anterior level consists of a small cluster of fusiform shaped cells dorso-medial to the dorsal aspect of the Mes. V (Swanson, 1976). The unimplanted, contralateral locus coeruleus of #66 is also shown in Figure 65. Note the absence of any distinct group of coeruleus neurons that is evident at mid levels of the locus coeruleus (e.g. Results, #106, Figure 3; Appendix I,
Figure 62, #52 and 77). From inspection of Figure 65 it can be seen that #66 self-stimulated at five different electrode sites each separated by 250 μm. At the most dorsal ICSS sites (#'s 1 and 2) the current thresholds were higher (36 μa and 30 μa) than at the most ventral sites (18 μa and 18 μa).

These observations suggest that the locus coeruleus is not the ICSS substrate in this region. If the locus coeruleus supported ICSS it would be expected that ICSS thresholds at sites just dorsal to the locus coeruleus (ICSS sites # 1 and 2, Figure 66) would be relatively low; this was not the case. More importantly, it might be expected that once the locus coeruleus had been damaged by lowering the electrode, ICSS thresholds should at least increase, if ICSS did not cease altogether. This clearly did not happen. Self-stimulation thresholds decreased despite the fact that the anterior pole of the locus coeruleus was destroyed. It would appear that some as yet unidentified non-noradrenergic neural system coursing in close proximity to the anterior pole of the locus coeruleus may be responsible for ICSS from this region. This possibility has recently been
suggested by Van Dop Kooy and Phillips (1977) who have attributed ICSS in the region of the locus coeruleus to activation of the nearby Mes. V.

An examination of ICSS sites in other regions of the pons and caudal midbrain further argues against involvement of NA systems in ICSS in these regions. Fluorescent histochemical examination of representative electrode placements scattered throughout the pons and caudal midbrain revealed that neither the magnitude nor the presence of ICSS was reliably related to the boundaries or density of NA systems. For example, rat #34 (Results, Figures 5-7) self-stimulated at sites dorsal to and within the PCS, where abundant NA fibers were seen leaving the locus coeruleus and coursing laterally in and through the PCS, presumably en route to the cerebellum (Ungerstedt, 1971a; Pickel et al., 1974). At each of the next seven electrode sites there was a slight lowering of ICSS thresholds. This occurred despite the fact that the NA density was greatest at the most dorsal sites in the PCS, and decreased considerably at the more ventral stimulation sites on the ventro-
medial edge of the Hot. V. At the tenth and most ventral site, animal #34 ceased to self-stimulate. A similar pattern was observed with rat #46 (Results, Figures, 5, 6, and 8). This animal failed to self-stimulate at any of the first six stimulation sites of the electrode penetration. Stimulation at site 7-9 yielded high rate, low threshold ICSS.

As shown in Figure 6, rat #46 first began to self-stimulate (position 1) when its electrode had passed through the dorsal tegmental NA bundle. As the electrode was lowered to positions 2 and 3, a total distance of 500 µm, the ICSS rates and thresholds remained constant. In this animal, ICSS first occurred and continued unabated, after the electrode had passed through the dorsal tegmental NA bundle.

Finally, rat #37 failed to self-stimulate even though the most ventrally tested electrode sites in this animal were amidst fibers of the dorsal tegmental NA bundle and central tegmental tract (Results, Figures 5 and 6). In fact, this animal vocalized and attempted to leap out of the test chamber at stimulation currents between 5-15 µA at each of the four most ventrally tested electrode sites.
The above examples illustrate that neither the presence nor the magnitude of ICSS is related to proximity to or density of NA fibers in the pons and caudal midbrain. While it is difficult to precisely quantify the density of NA neural elements in a given area, it was found (see Results) that ICSS current thresholds were not significantly correlated with the estimated density of NA neural elements at electrode sites sampled from five representative animals. These results and the failure to obtain ICSS from the locus coeruleus itself corroborate findings from several lesion studies (Clavier et al., 1976; Corbett et al., 1977; Cooper and Breese, 1976) which also suggested that NA systems did not mediate pontine tegmental ICSS. These latter conclusions were based on the failure of electrolytic (Corbett et al., 1977), or 6-hydroxydopamine (Clavier et al., 1976) lesions of the dorsal tegmental NA bundle or whole brain depletions of norepinephrine (Cooper and Breese, 1976) to disrupt ICSS from the region of the locus coeruleus. If NA systems are not involved in pontine tegmental and midbrain ICSS, what systems might be
supporting ICSS in these areas? Van Der Kooy and Phillips (1977) have suggested that activation of the trigeminal system may account for the ICSS previously attributed to the locus coeruleus. Their hypothesis rests on observations of ICSS from electrodes dorso-medial to and within the Mot. V. Gnawing is often associated with ICSS throughout the dorsal pontine tegmentum (Crow et al., 1972; Crow, 1976; Micco, 1974; Van Der Kooy and Phillips, 1977). The present findings are also consistent with the above observations. Self-stimulation was obtained from electrodes within and dorso-medial to the Mot. V (Results, Figure 5, #34; Figure 12, #35). As evident from Figure 1, ICSS was more readily obtained from electrodes that were lateral to the locus coeruleus than from electrodes within or medial to the locus coeruleus, an observation also made by Ritter and Stein (1973). It would appear that ICSS in the dorso-lateral pontine tegmentum is linked with activation of the trigeminal system. Van Der Kooy and Phillips (1977) offer the observation of a relation between gnawing and ICSS as evidence
that performance of a species-specific motor activity such as gnawing is in itself reinforcing, a view inherent in response-oriented theories of reinforcement (Glickman and Schiff, 1967; Kalenstein, Cox, and Kakolewski, 1970). The above view is inconsistent with observations in the present study of gnawing at sites that failed to support ICSS throughout the dorsal pons and ventral layers of cerebellum. It would appear that gnawing and ICSS are not related in all regions of the pons. Perhaps the relation between gnawing and ICSS reported by Van Der Kooy and Phillips (1977) reflects widespread and diffuse projections of the trigeminal system. Unfortunately, little is known about the central projections of the Mes V, other than its monosynaptic projections to the Mot. V (Darian-Smith, 1973). Another interpretation of these data is that trigeminal stimulation may contribute indirectly to ICSS by activating a behavior, gnawing, that is easily shaped towards a manipulandum such as a lever. Indeed, the lever-pressing response of pontine tegmental self-stimulation is characterized as much by gnawing of the lever as it is by pressing the
lever with the forepaws.

If the functions of the main sensory nucleus of the trigeminal and the Mes. V are to convey somatosensory and proprioceptive information from the head region (Darian-Smith, 1973), it would seem necessary to search for other neural systems which are perhaps more likely to support pontine tegmental ICSS. The most promising candidate system is one originating in the solitary nucleus that carries both visceral and gustatory information from the intermediate branch of the facial nerve, the glossopharyngeal, and the vagus nerves (Torvik, 1955, cited in Brodal, 1969). The second order neurons of this gustatory-visceral system project to the parabrachial nucleus. This nucleus is located just dorsal to and within the medial portion of the brachium conjunctivum or PCS (Norgren, 1978) at the level of the locus coeruleus. The second order fibers from the solitary nucleus project to and around the Mot. V, ventro-medial to and within the PCS, the Mes. V, but not to the locus coeruleus (Norgren, 1978). The third order neurons arising from the parabrachial nuclei ascend in the central tegmental tract largely
following the route of the dorsal tegmental NA bundle throughout the midbrain (Norgren, 1976).

Unlike the dorsal tegmental NA bundle, the thalamic third order gustatory-visceral fibers terminate in the ventrobasal part of the thalamus and various midline nuclei (Norgren, 1976) whereas fibers of the dorsal tegmental NA bundle mainly innervate the dorsal thalamic nuclei such as the anterior, paraventricular and rostromedial nuclei (Lindvall and Bjorklund, 1974; Jones and Moore, 1977). Both the tertiary gustatory-visceral fibers and the dorsal NA bundle fibers innervate the central amygdaloid nucleus (Norgren, 1976; Jones and Moore, 1977). Other areas traversed and perhaps receiving gustatory-visceral terminals are the subthalamic nucleus, which is nestled in the dorsomedial part of the internal capsule, the stria terminalis, the far-lateral area of the lateral hypothalamus and the substantia innominata (Norgren, 1976). From the above description it is apparent that ICSS sites in the pons and midbrain correlate just as well with the loci of the gustatory-visceral fibers as they do with the trigeminal system (Van Der Kooy and Phillips, 1977),
the dorsal NA system (Crow, 1972; Crow et al., 1972; Ritter and Stein, 1973), or the ventral NA bundle (Ritter and Stein, 1974). Indeed, it is rather surprising that Van Der Kooy and Phillips (1977) offered the trigeminal system such a paramount role in pontine tegmental ICSS, in view of the report from their own laboratory of ICSS from the region of the solitary nucleus (Carter and Phillips, 1975). As mentioned earlier, stimulation at many sites in the dorsal pontine tegmentum elicits licking responses which can be obtained independently of gnawing responses induced by trigeminal activation. It can be seen (Appendix I, Figure #'s 50, 57, 63 to 65) that a number of PCS, Mot. V, Mes. V and pericoeruleus placements found positive for ICSS encroached upon the parabrachial nucleus or areas traversed by the secondary and tertiary gustatory-visceral fibers originating from the solitary nucleus.

In conclusion, it seems that the solitary nucleus and its efferent projections appear to be the most promising candidate system for the support of ICSS in the pons and caudal midbrain. Additional
evidence in support of this view will be presented in discussing the relation between the DA systems and ICSS.

A second system from which ICSS may be obtained is found along the raphe of the caudal midbrain. Self-stimulation was reliably obtained from the dorsal raphe nucleus, particularly its mid-rostral aspect (Appendix I, Figure #43). Moreover, ICSS rates and current thresholds in the rostral dorsal raphe (Figure #13, 44-47) were comparable to those of the mid-lateral hypothalamus. Animals stimulated in the dorsal raphe nucleus exhibited extreme behavioral arousal, biting the lever, pressing in short bursts, and occasionally leaping from the test chamber. These behavioral responses were also common to animals with mid-lateral hypothalamic electrode placements. The dorsal raphe nucleus is the origin of the B7 serotonergic cell group (Dahlstrom and Fuxe, 1964). The dorsal raphe nucleus and surrounding area is innervated by fibers from both the locus coeruleus and the dorsal periventricular NA system (Lindvall and Bjorklund, 1974; Jones and Moore, 1977; Sakai, Salvert, Touret, and Jouvet, 1977). Since inhibition of serotonin synthesis by
pretreatment with the serotonergic synthesis inhibitor, parachlorophenylalanine (PCPA), increases rather than decreases dorsal raphe ICSS rates (Simon, LeMoal, and Cardo, 1976) it has been concluded that the catecholamine systems, rather than the serotonergic B7 cell group are responsible for dorsal raphe ICSS. The dorsal raphe nucleus contains some NA and DA cell bodies (Lindvall and Bjorklund, 1974; Saavedra, Groebecker, and Zivin, 1976) and projects to the neostriatum (Pasquier, Kemper, Forbes, and Morgane, 1977; Geyer, Puerto, Dawsey, Knapp, Bullard, and Mandell, 1976). It is not known whether the dorsal raphe-neostriatal pathway is serotonergic, DA or NA. Despite these facts it is not possible to rule out a direct role for serotonin in dorsal raphe ICSS since PCPA does not reduce tryptophan hydroxylase activity by more than 70-80% (Harvey and Gal, 1974). Thus, sufficient stores of serotonin may be synthesized to sustain ICSS for test periods of 30 min. as used by Simon et al. (1976). Support for this view stems from the work of Miliarissis, Bouchard, and Jacobowitz (1975) who observed that ICSS rates from the median raphe
nucleus or 88 serotonergic cell group (Dahlstrom and Fuxe, 1964) were not reduced by PCPA during the first 30 min. of the test session but declined dramatically over the remainder of the three hour test session. Longer test sessions have not as yet been used to study dorsal raphe ICSS. Whatever the neurochemical basis of dorsal raphe ICSS, it should be noted that it receives projections from the nucleus of the solitary tract (Aghajanian and Wing, 1977).

Another interesting observation made in the present study was the development of a behavioral pattern characterized by fearfulness that occurred after repeated stimulation over a number of test days with certain peri-coeruleus-electrode placements. Initially such animals exhibited grooming which was sometimes directed towards the lever. All of these animals however ceased to respond either after several minutes or several test sessions. Attempts to reinitiate responding by delivery of priming stimulations failed. These animals stopped approaching the lever and, with repeated testing, appeared to exhibit more and more
fear during the test sessions. This heightened fear was indicated by rapid respiration, increased evacuation, flattening of the ears and increased difficulty in handling. This general syndrome has not been reported previously in ICSS studies, but several researchers have noted that it is more difficult to obtain ICSS in the pontine tegmentum than at many limbic sites, as well as the abrupt cessation of ICSS responding from peri-coeruleus placements without apparent cause (Crow et al., 1972; Crow, 1976; Ritter and Stein, 1973). The difficulty of obtaining pontine tegmental ICSS might be caused by the activation of a neural system that induces a behavioral state of heightened emotionality. Such an idea has been suggested by Redmond and his colleagues (Redmond, Huang, Snyder, and Maas, 1976) who have reported that stimulation of the locus coeruleus in the stump-tailed monkey elicits a behavioral pattern similar to that elicited when these monkeys are exposed to sham threat from unfamiliar humans. Furthermore, lesions or pharmacological inactivation of the locus coeruleus reduce responsiveness to anxiety-inducing stimuli
(Redmond, Huang, and Gold, 1977, note #2). In support of this hypothesis, the anti-anxiety agent, diazepam, induces non-self-stimulating rats exhibiting heightened emotionality to initiate self-stimulation after a few priming stimulations (Corbett, 1977, note #3). It is tempting to attribute the "release of ICSS" to the anti-anxiety properties of diazepam and to further speculate that these anti-anxiety properties may result from the diazepam-induced inhibition of locus coeruleus neurons via action on presynaptic &-adrenergic receptors (Cedarbaum and Aghajanian, 1977; Aghajanian, Cedarbaum, and Wang, 1977). If activation of the locus coeruleus hinders or prevents the acquisition of ICSS in the dorso-lateral pontine tegmentum, then lesions of the locus coeruleus or the dorsal tegmental NA bundle might be expected to potentiate ICSS rates from other brain areas. Such seems to be the case with lateral hypothalamic ICSS sites. Rates of ICSS are potentiated by lesions of both the dorsal tegmental NA bundle (Corbett et al., 1977) and lesions of the locus coeruleus (Corbett, 1974, note #4; Koob, Balcom, and Meyerhoff, 1976).
It appears that the NA systems do not support ICSS and may in fact inhibit or interfere with ICSS. In contrast, the evidence from the present study and from numerous other studies in the literature strongly supports the view that activation of the midbrain DA systems underlies ICSS. Self-stimulation was reliably obtained in the region of the DA cell bodies surrounding both the interpeduncular nucleus and the ventral tegmental area of Tsai. As well, ICSS was reliably obtained from sites along the diencephalic route of the DA fiber systems. Moreover, it was found that ICSS current thresholds decreased and ICSS rates increased as electrodes were lowered towards the DA cell bodies and fiber systems. In fact, a strong correlation was found between ICSS current thresholds and the density of DA neural elements beneath the electrode tip. The A10 cell group, which gives rise to the mesolimbic and mesocortical DA systems (Ungerstedt, 1971a; Lindvall and Bjorklund, 1974; Lindvall et al., 1978; Thierry, Blanc, Sobel, Stinus, and Glowinski, 1973; Berger, Thierry, Tassin, and Mayne, 1976), consistently supported ICSS. By examining
Figures 22-30 (#128, #130, #101, and #139) it can be seen that the three animals (#128, #130, and #139) in which the electrode tips were in closest proximity to the A10 cell group have most similar ICSS rates and current thresholds. Rat #101 in which the electrode was somewhat more dorsally located than in the other three animals began at the most ventral position of the electrode to exhibit comparable ICSS rates and current thresholds. Animals #’s 69 and 70 (see Appendix I, Figures 90, 95, and 96) with similar electrode placements in the A10 area showed similar ICSS rates and current thresholds. Note that #69’s electrode was, if anything somewhat ventrally located with respect to #70’s electrode and was, therefore, somewhat closer to the A10 cell group. This difference may have resulted in #69 exhibiting slightly lower ICSS thresholds and higher ICSS rates than #70.

It is important to note, however, that the caudal portion of the A10 cell group did not support ICSS (Results, Figures 37 and 39; Appendix I, Figure 93 #113 and #115). It could be argued that
the failure to obtain ICSS from the caudal part of A10 reflects the less dense packing of the DA cell bodies in this region and that the stimulation would not activating sufficient numbers of DA cells to elicit ICSS. If this were the case, it would be difficult to explain the lack of ICSS by animal #115 (see Results, Figure 37) in which the electrode was in contact with and had been lowered through an area still rich in DA cell bodies and fibers. Moreover, in animals #113 and 115 stimulation currents up to 50 μA were ineffective at even the most ventrally tested electrode sites. At this very high current level it would be expected that the stimulation field, assuming a current spread of 0.5 - 1.0 mm, would include the caudal A10 cell group as well as the A8 cell group. Despite the use of increased current, only #115 displayed approach behavior. This animal would orient towards the lever and show some sniffing behavior at a current intensity of 50 μA. The electrodes were not faulty in these animals; stimulation at current intensities below 25 μA elicited a variety of responses including eye blinks, rolling of the head to one side, etc...
Interestingly, the most dorsal of the electrode sites in these animals yielded extreme escape reactions. All three animals leapt from the test chamber at currents ranging from 15-20 μa. Prior to the animals leaping from the test chamber, pupillary dilation, increased respiration and rapid contraction of the anal musculature was observed. These electrode sites appeared to border the most caudal part of the red nucleus, pars magnocellularis. Aversive stimulation sites in or near the red nucleus have been noted previously (Olds and Olds, 1963). Other investigators (Dreese, 1966a; Olds and Olds, 1963; Huang and Routtenberg, 1971; Crow, 1972; Prado-Alcala et al., 1975) who have mapped the A10 region for ICSS have only mapped the mid-rostral aspect of the A10 cell group. Thus, the present data suggest that ICSS may not be equally supported at all levels of the A10 cell group.

The role of the A9 cell group in ICSS is unclear from the present data. Part of the uncertainty arises from the lack of a distinct boundary between the A9 and A10 cell groups, a feature that has led some
(e.g., Moore and Bloom, 1978) to drop the terms A8, A9, and A10 and to speak only of mesotelencephalic DA systems. However, in order to relate the data in the present study to those of other ICSS mapping studies, the A8, A9, and A10 distinctions will be retained. The A10 area is defined as the area above the interpeduncular nucleus and extending laterally to the most medial part of the zona compacta. Thus the ventral tegmental area of Tsai is included within the A10 area, while the A9-cell group conforms to the zona compacta. At the rostral level of the interpeduncular nucleus, ICSS was found just lateral to the ventral tegmental nucleus on the medial edge of A9 (see Results, Figures 26, 31, and 34, #100). Even more rostrally, at the level of the mammillary bodies, ICSS was obtained from the midlateral region of A9 (see Results, Figures, 26, 33, and 36, #102). Note that there was a sudden drop in ICSS current threshold when the electrode was moved from ICSS site #1 (31 μa) to ICSS site #2 (22 μa). This drop in ICSS threshold coincided with the electrode being lowered through the ventral portion of the medial lemniscus. More caudal A9 electrode placements gave
mixed results. Four animals (#103, #117, #129, and #132) with electrodes located in the mid-lateral part of A9 failed to exhibit ICSS. Three others, however, (#58, #134, and #138) all with A9 placements, displayed ICSS. The electrode in rat #58 (see Appendix I, Figure 81) was somewhat medially located, within approximately 500 µm of the ventral tegmental nucleus. Thus it could be argued that the ICSS observed in this animal was the result of current spread to the medially located A10 area. Such an explanation could not account for the ICSS observed in animals #134 and #138. The electrode placements of these animals were similar to the electrode placements of the four animals discussed above (#103, #117, #129, and #132) none of whom self-stimulated. Careful examination of the electrode placement in animal #134 (Appendix I, Figure 90) reveals that the electrode had just passed through the zona compacta into the zona reticulata. This animal self-stimulated at the most dorsally tested electrode site (i.e. the zona compacta) but abruptly ceased responding when the electrode was lowered 250 µm (see Appendix I, Figure 93). This sudden
cessation of responding may not be so surprising when one considers the fact that the zona compacta in the rat is at most 250 μm thick in the horizontal plane. This latter observation is further complicated by the recent report (Fallon, Riley, and Moore, 1978) of dorsal-ventral subdivisions within the zona compacta itself. A dorsal sheet contains fusiform shaped cells with dendrites restricted to the zona compacta. These cells project to allocortical regions of the basal forebrain such as the olfactory tubercle and amygdala. The cells of the ventral sheet project to the striatum and are pyramidal in shape, with dendrites directed towards the zona reticulata. These two cell types can be distinguished in any of the A9-A10 montages. Thus, the failure to obtain ICSS from certain A9 placements (e.g. #129, Figure 90, Appendix I) could be the result of the electrodes piercing one or both layers of the zona compacta on implantation. Another factor contributing to the failure to obtain ICSS from laterally located A9 placements (e.g. #117, see Results, Figures 39 and 41) is that the fibers of the A9 cell group tend to run medially towards the
ventral tegmental area, away from laterally located electrode placements (Lindvall and Bjorklund, 1974).

In view of the number of reports implicating the A9 cell group and the nigrostriatal system in ICSS (Crow, 1972; Prado-Alcala et al., 1975; Phillips et al., 1976a; German and Bowden, 1974) it might seem surprising to question these findings. It is the case, however, that most of the positive A9 placements reported in the above studies (Crow, 1972; Prado-Alcala et al., 1975; also see German and Bowden, 1974) actually border upon the A10 area. Reports of ICSS from the caudate nucleus (Olds and Olds, 1963; Routtenberg, 1971; Phillips et al., 1976a) have been interpreted as providing support for the notion that the A9 nigrostriatal system supports ICSS (Phillips et al., 1976a; Crow, 1976; German and Bowden, 1974). These data must be reconsidered in light of evidence describing a projection of the A10 cell group to the caudate nucleus (Domesick, Beckstead, and Nauta, 1976, note #5). Also, there is evidence that cells of the dorsal raphe nucleus project to the caudate nucleus (Miller, Richardson, Fibiger, and McLennan, 1975;
Geyer et al., 1976; Pasquier et al., 1977).
It is interesting to note that the dorsal raphe projection is to the medial and ventro-caudal part of the caudate (Pasquier et al., 1977). Self-stimulation rates from this part of the caudate nucleus are greatly reduced following pretreatment with PCPA (Phillips et al., 1976b).

In summary, the present data suggest that while some A9 placements (anterior-medial) support ICSS other A9 placements (posterior-lateral) do not. It is not possible to decide whether these discrepancies reflect functional differences within the A9 cell group or complexities of the anatomy of this area (Fallon et al., 1978) not previously appreciated.

It appears from the present study that the A8 cell group does not support ICSS. This finding is in agreement with those of Huang and Routtenberg (1971) who failed to obtain ICSS from the area now known to contain the A8 cell group. This cell group, unlike the A9 and A10 cell groups does not project to the neocortex, rather, its primary projection seems to be to the lateral part of the caudate nucleus (Lindvall et al., 1978).
In the hypothalamus, ICSS was obtained along the route of the medial forebrain bundle as it courses through the lateral hypothalamus. High ICSS rates and low current thresholds were obtained just medial to and within the tip of the internal capsule (see Results, Figures 19 and 20, Plates -0.2, -0.8, and -1.8). This latter area is traversed by the nigrostriatal, mesolimbic and mesocortical DA systems (Ungerstedt, 1971a; Lindvall and Bjorklund, 1974). An example of an electrode placement on the medial edge of the internal capsule is shown in Figure 71 (see Appendix I). In this animal (#87) ICSS was obtained from five different electrode sites (Appendix I, Figure 76). Since the electrode was lowered in steps of 250 μm, the most dorsal ICSS site would probably have been in the zona incerta just dorsal to the internal capsule. Note the very high ICSS rates displayed by this animal and the gradual increase in current threshold when the electrode was moved from the most dorsal ICSS site to the most ventrally tested ICSS site.
High rates of ICSS and low current thresholds were also found at electrode sites medial to the DA fibers. A perifornical electrode placement, medial and caudal to the electrode placement described above, is shown in Figure 81 (Appendix I). Self-stimulation was obtained at five electrode sites in this animal (#111, Figure 82). The most dorsal ICSS site was probably located mid-way between the fornix and the mamillothalamic tract. This animal could not be tested at current intensities greater than 22 µa at any of the five ICSS sites. At higher currents, the animal would leap from the test chamber. At ICSS test sites #2-5, current intensities above 20 µa could not be used for the same reason. While it is true that ICSS can be obtained from sites traversed by DA fibers in the lateral hypothalamus and internal capsule, it is unlikely that the laterally located DA systems could underly medial, perifornical hypothalamic ICSS. This last point raises several important questions regarding the role of the dopamine systems in ICSS.

First, it seems reasonable to ask, is activation of DA systems a necessary prerequisite for obtaining
ICSS? It seems clear that not all ICSS can be accounted for by the direct activation of DA systems, yet ICSS from non-DA areas in the dorsal pontine tegmentum is eliminated following pretreatment with the DA receptor blocker, pimozide (Corbett, Yokel, and Fouriezos, 1975, note #6). The finding that ICSS at non-DA sites is eliminated by manipulations that reduce DA synaptic function has led some (e.g., Cooper, Konbol, and Breese, 1978) to conclude that dopamine is not involved in the rewarding aspects of ICSS but in the control of motor integration. This view is contradicted by the results of pharmacological studies that have to some extent dissociated reward and performance deficits (Fouriezos and Wise, 1976; Fouriezos, Hansson, and Wise, 1978, note #7).

Perhaps ICSS from sites not containing DA neurons can be accounted for by the indirect activation of the DA systems across one or more synapses. While there is anatomical evidence indicating monosynaptic connections between certain non-DA ICSS sites such as the dorsal raphe nucleus and the midbrain DA cell group (Conrad, Leonard, and
Pfaff, 1974; Bunney and Aghajanian, 1976; Pasquier et al., 1977; Moore, Halari, and Jones, 1978; Azmitia and Segal, 1978) no monosynaptic connections have as yet been described linking ICSS sites in cerebellum, hippocampus and pons with the midbrain DA systems. In fact, the innervation of the midbrain DA cell groups by caudally originating fibers is surprisingly sparse (Bunney and Aghajanian, 1976). Of course, currently available anatomical tracing techniques reveal only monosynaptic projections.

Self-stimulation has been demonstrated in both the thalamic animal (Huston, 1975) and in the precollicular, hemi-decerebrate animal (Huston and Ornstein, 1977, note #8). Such results suggest that ICSS may exist independently of brain DA systems. The results of the present study as well as data discussed earlier (Corbett et al., 1977; Clavier et al., 1976; Cooper and Breese, 1976) seem to exclude a role for NA systems in ICSS. The serotonergic systems originating in the dorsal and median raphe nuclei both seem capable of supporting ICSS. Self-stimulation has been obtained from the
nuclei of origin of these systems (Margules, 1969; Miliaressis et al., 1975; Simon et al., 1975, 1976) as well as along their efferent trajectories, and from their terminal areas (Phillips et al., 1976a). The trajectories and terminal sites of the serotonergic systems are for the most part coextensive with the trajectories and terminal areas of the DA systems (Conrad et al., 1974; Moore et al., 1978; Azmitia and Segal, 1978). Moreover, ICSS in the hippocampus (an area not innervated by DA systems), the median raphe nucleus, and the caudate nucleus has been shown to be disrupted by serotonin depletion (Miliaressis et al., 1975; Phillips et al., 1976b; Van Der Kooy, Fibiger, and Phillips, 1977).

Activation of the serotonergic systems that course medially to the DA systems throughout the hypothalamus (Conrad et al., 1974; Moore et al., 1978; Azmitia and Segal, 1978) may in part underlie the medial or perifornical hypothalamic ICSS observed in the present study.

It was argued earlier that gustatory-visceral fibers originating in the nucleus solitarius (Norgren, 1978) could perhaps account for the ICSS
in the dorsal pons. Although ICSS sites in the pons and midbrain appear to follow the trajectory of the gustatory-visceral fiber systems, there is more compelling evidence to implicate this system in ICSS. Rolls, Burton, and Mora (1976, 1977) have described neurons in the far lateral area of the lateral hypothalamus and in the substantia innominata that alter their firing rate in response to food. Some neurons responded best to water placed on the tongue and not at all to a sucrose solution. The activity of these neurons was not affected by olfactory stimuli nor by volitional movement. Furthermore, the magnitude of the neural response correlated with food preference; a greater inhibition or excitation occurred in the presence of highly preferred foods. These neurons did not respond when the animal was satiated. Other neurons particularly in the lateral hypothalamus did not respond to the taste of food, but instead to the sight of food (Rolls et al., 1976, 1977). The substantia innominata is an area lying on the edge of the optic tract, just ventral to the internal capsule (Nauta and Haymaker, 1969). Of
particular interest are the afferent projections to this area. Third order gustatory-visceral fibers pierce and innervate the medial tip of the internal capsule, subthalamic nucleus, lateral hypothalamus and substantia innominata (Norgren, 1976).

All of these areas are also heavily innervated by DA and serotonergic fiber systems (Ungerstedt, 1971; Lindvall and Bjorklund, 1974; Fallon and Moore, 1978; Conrad et al., 1974; Moore et al., 1978; Azmitia and Segal, 1978). Finally, the substantia innominata receives direct input from the habenular nuclei (Herkenham and Nauta, 1977b). The habenular input is of interest because it has recently been reported by Sutherland and Nakajima (1978, note #9) that the habenular nucleus and its efferent pathway, the fasciculus retroflexus support ICSS. Furthermore, they found that lesioning of the midbrain raphe nuclei in one animal abolished ICSS from an electrode that was located in the fasciculus retroflexus. The habenular nuclei are reciprocally connected with the dorsal and median raphe nuclei, the ventral tegmental area of Tsai and the interpeduncular nucleus. To summarize, a dorsal and a ventral pathway are seen
linking the midbrain DA and serotonergic systems with the substantia innominata and surrounding basal olfactory area. Both of these pathways are coextensive with, or under the influence of ascending gustatory-visceral pathways (Norgren, 1976). It seems likely that the DA and serotonergic systems are so organized as to interact with gustatory-visceral and olfactory systems in the substantia innominata and lateral hypothalamus. It would be interesting to investigate the nature of this interaction and in so doing turn to a study of the role of sensory factors underlying ICSS and reward processes in general. The work of Rolls and associates (Rolls et al., 1976, 1977) suggests that such an approach might be fruitful.

Implicit in the above discussion is the notion that DA and perhaps also serotonergic systems may have modulatory functions in ICSS. This raises the second question, and that is, given that DA systems are involved in ICSS, what is the nature of this involvement? It is clear from drug self-administration studies that increasing DA synaptic function produces rewarding effects (Pickens and Harris, 1968;
Baxter, Gluckman, Stein, and Scerni, 1974; Yokel and Wise, 1975) that presumably contribute to ICSS at sites rich in DA neurons. Attention to particular environmental stimuli (selective attention) seems to be directly or intimately associated with DA synaptic function. That is, animals including man exhibit reduced orientation and attention to environmental stimuli when brain DA levels are greatly reduced (Ungerstedt, 1971b; Sachs, 1976). Increased DA synaptic function results in increased attention to particular environmental stimuli (Robbins, 1976). The two behavioral extremes of reduced and increased DA synaptic function have been termed sensory inattention (Marshall et al., 1974) and stereotypy (Randrup and Munkvad, 1970). Indeed, ICSS in the region of the midbrain DA systems appears to be more stereotyped or locked in than medial hypothalamic, dorsal raphe, and especially pontine tegmental ICSS. Self-stimulation is acquired very rapidly at most sites containing abundant DA cell bodies and fibers (caudate ICSS being an important exception), shaping is not necessary, and the ICSS once obtained is reliable
and not easily disruptible by external stimuli. In contrast, ICSS in the pons is acquired slowly, shaping is usually necessary, and the ICSS seems particularly sensitive to disruption by external stimuli. Also, ICSS at these sites seems to lack the compulsive or locked in character that typifies ICSS from DA ICSS sites. It may be that ICSS at pontine tegmental sites has a primarily sensory substrate, such as the ascending gustatory-visceral fiber systems. Lateral hypothalamic ICSS on the other hand may result from the direct activation of DA fibers, serotonergic fibers, as well as specific sensory systems (olfactory and gustatory-visceral). There is a great deal of evidence to support this view. Lateral hypothalamic ICSS has been extremely resistant to disruption by any but the most massive lesions (Valenstein, 1966; Lorens, 1976). The reduction of whole brain DA levels to less than 19% of control levels initially eliminates lateral hypothalamic ICSS but the ICSS rates recover over a period of six weeks to approximately 50% of the prelesion rate of responding (Phillips and Fibiger, 1976).

Recovery of ICSS from the region of the A9 and A10 cell
groups was complete 8-10 days after 6-
hydroxydopamine induced lesions of the ascending
DA fiber systems despite striatal dopamine levels
being lowered to less than 3% of control values
(Clavier and Fibiger, 1977). Since the 6-
hydroxydopamine was injected into the hypothalamus,
extensive damage to the mesocortical and mesolimbic
DA systems would also be expected. Unfortunately,
Clavier and Fibiger (1977) only measured striatal
dopamine levels, although they mention that similar
lesions produce approximately 90% depletions of
mesocortical and mesolimbic dopamine levels.

The above data suggest that other, non-DA
systems are involved in lateral hypothalamic and
substantia nigra ICSS. It could be argued that
the remaining stores of dopamine and the development
of DA receptor supersensitivity (Ungerstedt, 1971c)
could account for the failure to completely
eliminate lateral hypothalamic (Phillips and
Fibiger, 1975) and substantia nigra (Clavier and
Fibiger, 1977) ICSS. It is not possible to refute
this interpretation since it is unlikely that any
lesion would completely deplete all brain dopamine
stores and even if this were possible, the animals probably would not survive. It should be remembered that some of the brain dopamine remaining after 6-hydroxydopamine lesions is dopamine existing in precursor form in NA neurons. Thus the actual levels of brain DA in surviving dopamine neurons is probably less than the published values. A more reasonable interpretation of these data is that expressed above, namely, that the DA systems are capable of supporting ICSS, but more importantly they modulate or bias all goal-directed behavior. This modulation may be shared with other putative neurotransmitters such as serotonin and the endorphins. Self-stimulation at different sites would depend to varying degree upon DA systems. Thus, the failure to eliminate lateral hypothalamic or substantia nigra ICSS may indicate the presence of multiple non-DA systems which contribute to ICSS in these regions. As was discussed earlier, the lateral hypothalamic-ventral tegmental areas are linked dorsally and ventrally by a variety of inputs of olfactory and gustatory-visceral origin (Nauta and Haymaker, 1969; Norgren, 1976, 1978; Herbenham and
Nauta, 1977a,b). Any of these systems may contribute to ICSS. Indeed, lateral hypotalamic stimulation triggers a variety of cephalic reflexes (Powley, 1977) and it has been reported that vagotomy decreases ICSS rates from certain hypothalamic electrode sites (Ball, 1974). Also, lesions caudal to the location of the DA cell bodies have been shown to disrupt ICSS from the substantia nigra (Belluzzi, Ritter, Wise, and Stein, 1975) and this effect is not due to interruption of the ascending NA fiber systems (Phillips, 1975; note #10). Self-stimulation at sites in the tip of the internal capsule is greatly reduced following lesions that encroach upon the ascending gustatory-visceral fiber systems (Farber et al., 1976).

It would appear that non-DA and non-serotonergic systems participate in ICSS from sites (lateral hypotalamus, internal capsule) thought to have a DA substrate (German and Bowden, 1974; Crow, 1976). In view of these data and the findings reported in this thesis, it seems that the catecholamine hypothesis of reward (German and Bowden, 1974;
Crow, 1976, 1977) may no longer be useful for
directing future research.

In conclusion, the locus coeruleus and other
pontine NA systems were found not to support ICSS.
Instead, a system of gustatory-visceral fibers is
suggested as a candidate system for ICSS in these
areas. This system may also contribute to ICSS
at hypothalamic and other limbic areas (e.g.
substantia innominata). Self-stimulation was readily
obtained from the A10 DA cell group and at sites
in the hypothalamus that are traversed by the
midbrain DA systems. Results with the A9 cell group
were equivocal; some sites supported ICSS, others
did not. This may reflect a functional division
within the A9 cell group or it may reflect the now
apparent anatomical complexity of the DA systems
(Lindvall and Björklund, 1974; Lindvall et al., 1978;
Moothe and Bloom, 1978). The caudal DA cell group
A8 and caudal pole of A10 did not support ICSS.
An argument was made for the multiplicity of the reward
circuitry with DA systems being ascribed an important,
but not exclusive role. Given the common origin
of the substantia nigra and the midbrain raphe nuclei
(Lenn, Hallow, and Rakic; 1978) and their connections with the habenular nuclei (Herkenham and Nauta, 1977a,b), it would seem best to view these monoamine systems as components of a midbrain limbic system, rather than as reward systems existing in splendid isolation.
REFERENCE NOTES


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Figure 43. Thionin stained sections of the electrode tracts of animals 1, 2, 3, 4, 6, 7, 8, and 10. Electrode tip is indicated by an arrow. Abbreviations: DR = dorsal raphe nucleus; DTG = dorsal tegmental nucleus of Gudden; PAG = periaqueductal gray; PCS = superior cerebellar peduncle.
Figures 44-49. Self-stimulation current threshold and rate-intensity data of animals 1, 4, 6, 7, 8, and 10. The interval between each self-stimulation site was 125 μm. The data are illustrated as described for Figures 7-11.
Figure 50. Thionin stained sections of the electrode tracts of animals 11, 12, 13, 15, 18, 19, 26, 30, and 32. Electrode tip is indicated by an arrow. Abbreviations: LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve; Mot. V = motor nucleus of the trigeminal nerve; PCS = superior cerebellar peduncle.
Figures 51-56. Self-stimulation current threshold and rate-intensity data of animals 12, 13, 18, 19, 26, and 30. The interval between each self-stimulation site was 125 μm. The data are illustrated as described for Figures 7-11.
Figure 57. Thionin stained sections of the electrode tracts of animals 16, 27, 36, 54, 75, 81, 104, 119, and 120. Electrode tip is indicated by an arrow. Abbreviations: LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve; Mot. V = motor nucleus of the trigeminal nerve; MR = median raphe nucleus; PCS = superior cerebellar peduncle.
Figures 58-62. Self-stimulation current
threshold and rate-intensity data of animals
16, 27, 75, 119, and 120. The interval between
each self-stimulation site was 125 μm for animals
16 and 27, and 250 μm for animals 75, 119, and 120.
The data are illustrated as described for Figures
7-11.
Figure 63. Thionin stained sections of the electrode tracts of animals 25, 29, 31, 50, 52, 76, 77, 78, and 79. Electrode tip is indicated by an arrow. Abbreviations: LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve; PCS = superior cerebellar peduncle; VII = facial nerve.
Figure 64. Self-stimulation current threshold and rate-intensity data of animal 25. The interval between each self-stimulation site is 25 µm. The data are illustrated as described for Figures 7-11.
Figure 85. Thionin stained sections of the electrode tracts of animals 66, 82, 107, 121, 122, and 141. Electrode tip is indicated by an arrow. Abbreviations: CER = cerebellum; IV = fourth ventricle; LC = locus coeruleus; Mes. V = mesencephalic nucleus of the trigeminal nerve.
Figures 66-69. Self-stimulation current threshold and rate-intensity data of animals 66, 121, 127, and 141. The interval between each self-stimulation site is 250 µm. The data are illustrated as described for Figures 7-11.
Figures 70-71. Thionin stained sections of the electrode tracts of animals 39, 42, 43, 53, 55, 105, and 118 (Figure 70) and of animals 59, 60, 62, 87, 88, 89, 97, 98, and 110 (Figure 71). Electrode tip is indicated by an arrow. Abbreviations:

CER = cerebellum; FX = fornix; IC = internal capsule;
IV = fourth ventricle; LC = locus coeruleus;
Mes. V = mesencephalic nucleus of the trigeminal nerve; MT = mamillothalamic tract.
Figures 72-80. Self-stimulation current threshold and rate-intensity data of animals 59, 62, 97, 60, 87, 88, 89, 98, and 110. The interval between each self-stimulation site is 250 μm. The data are illustrated as described for figures 7-11.
Figure 81. Thionin stained sections of the electrode tracts of animals 57, 58, 86, 90, 109, 111, 112, 125, and 126. Electrode tip is indicated by an arrow. Abbreviations: FR = fasciculus retroflexus; FX = fornix; IC = internal capsule; ML = medial lemniscus; MT = mamillothalamic tract; SNR = substantia nigra, pars reticulata; STN = subthalamic nucleus; VTN = ventral tegmental nucleus of Tsai; ZI = zona incerta.
Figures 82-89. Self-stimulation current thresholds and rate-intensity data of animals 111, 125, 57, 90, 107, 86, 126, and 58. The interval between each self-stimulation site is 250 \( \mu \text{m} \). The data are illustrated as described for Figures 7-11.
Figure 90. Thionin stained sections of the electrode tracts of animals 68, 69, 70, 83, 84, 85, 127, 129, and 134. Electrode tip is indicated by an arrow. Abbreviations: LP = interpeduncular nucleus; ML = medial lemniscus; PAG = periaqueductal gray; RN = red nucleus; SNR = substantia nigra, pars reticulata; VTN = ventral tegmental nucleus of Tsai.
Figures 91-97. Self-stimulation current thresholds and rate-intensity data of animals 83, 127, 134, 68, 69, 70, and 84. The interval between each self-stimulation site is 250 μm. The data are illustrated as described for Figures 7-11.
Figure 98. Thionin stained sections of the electrode tracts of animals 99, 113, 115, 116, 136, 197, and 138. Electrode tip is indicated by an arrow. Abbreviations: IP = interpeduncular nucleus; ML = medial lemniscus; RN = red nucleus; SNC = substantia nigra, pars compacta.
Figures 99-100. Self-stimulation current thresholds, and rate-intensity data of animals 99 and 138. The interval between each self-stimulation site is 250 μm. The data are illustrated as described for Figures 7-11.
Ratings of NA Fluorescence Intensity
High = 1; Medium = 2; Low = 3

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